CLAIM LISTING

1. (previously presented) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) provide an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least first and second subpopulations, distributed at discrete sites on a surface of a substrate;

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to said first subpopulation;

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to said second subpopulation;

d) simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primer, respectively;

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively; and

f) determining the identity and location of each microsphere.

2. (previously presented) A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. (previously presented) A method according to claim 1 wherein at least said first sequence primer is attached to said first microsphere.

4. (previously presented) A method according to claim 1 wherein said first and second hybridization complexes comprise:

a) said first and second target sequences;

b) said first and second sequence primers;

c) first and second capture probes, wherein said capture probes are covalently attached to said first and second microspheres, respectively.

5. (previously presented) A method according to claim 1, wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequence primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. (previously presented) A method according to claim 1 further comprising:

d) extending said first and second extended primers by the addition of a second nucleotide to the second detection position using said first enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. (original) The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

8. (original) A method according to claim 7 wherein said second enzyme is sulfurylase.

9. (original) A method according to claim 7 wherein said third enzyme is luciferase.

10. (previously presented) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of said first target sequence,

b) providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of said second target sequence, wherein said first and second sequencing primers are covalently attached to microspheres distributed at discrete sites on a surface of a substrate;

b) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second sequencing primers, respectively.

11. (previously presented) A method according to claim l0 wherein said first hybridization complex and said second hybridization complex each comprise a capture probe.

12. (previously presented) A method according to claim 10 wherein said capture probe is a sequencing primer.

13. (previously presented) A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. (original) The method according to claim 13 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

15. (original) A method according to claim 14 wherein said second enzyme is sulfurylase.

16. (original) A method according to claim 14 wherein said third enzyme is luciferase.

17. (previously presented) A method according to claim 10 wherein said determining comprises:

a) providing a sequence primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said first enzyme; and

f) determining the identification of said second protected nucleotide.

18. (previously presented) A kit for nucleic acid sequencing comprising:

a) a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres distributed on said sites;

wherein said microspheres comprise capture probes;

b) a first extension enzyme; and

c) dNTPs.

19. (original) A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP.

20. (original) A kit according to claim 18 wherein said dNTPs are labeled.

21. (original) A kit according to claim 20 wherein each dNTP comprises a different label.

22. (previously presented) The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.

23. (previously presented) The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. (previously presented) The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. (previously presented) The method according to claim 10, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. (previously presented) The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. (previously presented) The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. (previously presented) The kit according to claim 18, wherein discrete sites are wells.

29. (previously presented) The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. (previously presented) The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.

31. (previously presented) The method according to claim 1, wherein said microsphere array is decoded prior to providing first and second hybridization complexes.

32. (previously presented) The method according to claim 31, wherein said microspheres further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identity and location of each microsphere can be determined.

33. (new) A method according to claim 11 wherein said first hybridization complex and said second hybridization complex further comprise an adapter probe.

34. (new) A method of sequencing a genome comprising:

a) amplifying a genome, thereby obtaining a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions;

b) providing an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and second subpopulation, distributed at said discrete sites;

c) hybridizing sequencing primers to said first domains of said target sequences, wherein said hybridization complexes are attached to said microspheres;

d) simultaneously extending said primers by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primers; and

f) determining, the identity and location of said microspheres.

REMARKS

Claims 1 to 34 are presently pending and under examination.

REJECTIONS UNDER 35 U.S.C. § 103 (a)

Applicants respectfully traverse the rejection of claims 1-4, 6-10, 12-17, and 22-27 as allegedly obvious under 35 U.S.C. § 103(a) over Navot et al., United States Patent No.6,335,165, and Walt et al., United States Patent No. 6,327,410.

In response to Applicants' arguments mailed November 3, 2003, the current Office Action, starting at page 2, final paragraph, asserts that there would have been motivation to combine the cited Navot et al. and Walt et al. patents. The Office Action asserts that Navot et al. teach pyrosequencing of nucleic acids which may be attached to microbeads in an